PATERNITY TESTING BY OLIGONUCLEOTIDE DNA FINGERPRINTING: A MULTI-CENTER STUDY PROVING RELIABILITY AND VALIDITY

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INTRODUCTION

While Jeffreys and co-workers (1) demonstrated in an extensive study of 1702 paternity cases that paternity testing with multi-locus (ML) probes 33.6 and 33.15 is effective and reliable, this technique nevertheless remained an issue of controversial debate.

The aims of our multi-center study (2), comprising 256 paternity cases submitted to 7 different German laboratories was: (i) to assess whether or not the positive results obtained for Jeffrey's probes also hold true for the multi-locus oligonucleotide probe $(CAC)_5/(GTG)_5$, (ii) to evaluate the robustness of paternity testing against differing laboratory protocols and (iii) to determine the extent of correspondence between observed data and parameters expected under analytical models, e.g. whether the assumption of statistical independence is valid for electrophoretical bands on the same gel.

MATERIAL AND METHODS

DNA was digested with restriction enzyme HinfI and hybridized with oligonucleotide $(CAC)_5/(GTG)_5$ (Fresenius, Oberursel). Transformation of entire ML banding patterns into series of position-wise phenotype patterns (e.g. band present in the patterns of mother and child, but not for the alleged father: M+C+F-, or M-C+F+, M+C+F+, etc.) followed the procedure as described in (2). Only fragments larger than 4 kb were evaluated. Paternity probabilities were calculated from the likelihood ratios of paternity vs. non-paternity which, in turn, are based on the observed numbers of the different, position-wise phenotype patterns (3).

RESULTS

All parameters characteristic of ML DNA fingerprints were found to differ significantly between the contributing centres, e.g. the number of analyzed gel positions, the number of bands scored per individual, the probability of occurrence of a band at a particular position of a gel, and the band-sharing probability between the mother and both the child and the alleged father.

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Nevertheless, paternity cases could be clearly divided into two subgroups on the basis of (i) the proportion of offspring bands that could not be assigned to either parent, and (ii) the extent of band sharing between child and alleged father. This partitioning, which most likely corresponds to true and false paternity, confirms previous findings obtained for Jeffrey's ML-probes (1) and can be achieved without biometrical analysis.

The assumption of statistical independence of gel positions in the formal analysis of ML-patterns has been shown to be not always justified. However, allelism and linkage between loci are of only minor importance for the following reasons. Loci contributing to DNA fingerprints generated with probe $(CAC)_5/(GTG)_5$ are evenly scattered throughout the genome (4), and the majority of these comprise a large number of alleles. However, only a minor fraction of alleles, representing also the rarer ones, is larger than 4kb (5) and therefore actually scored. Thus, only a small number of the evaluated DNA fragments can be allelic.

Finally, theoretical considerations and simulations (3) indicate that partial homozygosity, allelism and linkage do not alter paternity probabilities to an extent relevant for decision making, i.e. in the range of 0.1% and 99.9%. It should be noted in this context, that confining the numerical analysis to large fragments also renders invalid one of the major arguments put forward against the likelihood approach, namely that ML DNA fingerprints are merely overlays of single-locus patterns (6)

Under the above assumptions, position-wise phenotypes are approximately independent from each other, and the number of bands per individual should consequently follow a Bernoulli distribution. However, the empirical distribution of the normalized number of bands per individual differs slightly from it's standard normal expectation although no systematic deviation is apparent. Further, all position-wise phenotype patterns, as introduced above, have been observed at frequencies not dissimilar to their theoretical expectations. This result not only supports the likelihood approach but specifically justifies the highly debated inclusion of positions lacking a band in mother, child and alleged father (pattern M-C-F-) in the calculation of paternity probabilities. Such positions could have been occupied by a band in the pattern of the alleged father, and since not transmitted, would have argued against paternity.

The distribution of $\log_{10}$-likelihood ratios (LR) allows a clear-cut distinction between two groups of cases. A total of 43 cases yielded LR-values smaller than zero, with a maximum value of -3.9288 (which corresponds to a paternity probability of 0.02% according to Essen-Möller). The remaining cases are characterized by LR values larger than 1.9 (Paternity probability of 98.87%). This partitioning coincides perfectly with that described above. Variations of LR between laboratories can be explained by different laboratory protocols, beeing reflected especially in different numbers of gel positions scored.

CONCLUSION

Our study demonstrates that paternity testing with the ML-oligonucleotide probe $(CAC)_5/(GTG)_5$ is reliable and allows decision making that is robust against methodological variations. Rare, undecisive cases may nevertheless occur and should be solved by alternative means, e.g. single-locus testing. We also demonstrated that the major arguments put forward against the likelihood approach in quantitative paternity testing are invalid for this ML system.

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